Description of the eggs and yolk-sac larvae of Whitebait Hyperlophus vittatus (Teleostei: Clupeidae)

R.J. Tregonning, D.J. Gaughan and W.J. Fletcher

Bernard Bowen Fisheries Research Institute, Western Australian Marine Research Laboratories, P.O. Box 20, North Beach, Western Australia 6020, Australia

Abstract – The eggs and early larval (yolk-sac) stages of *Hyperlophus vittatus* are described. The eggs are spherical, range from 0.83 - 0.95 mm in diameter (mode = 0.93 mm), have a perivitelline space which occupies 8.6 - 30.9% (mode = 14.5%) of the diameter, a coarsely and completely segmented yolk and one oil globule (0.025 - 0.075 mm in diameter, mode = 0.048 mm). The eggs hatch in 2 - 3 days at a mean temperature of 17° C. The yolk-sac larvae are approximately 2.6 mm BL at hatching and lack pigmented eyes or a functional mouth. The larvae are elongate and have a long, straight gut (pre-anal length = 70 - 79% of BL) with the single oil globule located posteriorly in the yolk-sac. The presence of melanophores immediately dorsal of the posterior tip of the notochord distinguish the yolk-sac larvae of *H. vittatus* from those of other clupeids in southern Western Australia.

INTRODUCTION

Whitebait (Hyperlophus vittatus Castelnau), or sandy sprat, their common name elsewhere in Australia, is commercially fished in the southwest of Western Australia (Goh 1992). The confinement of whitebait fishing in this region to beach seining operations has restricted the amount of information available on the distribution of this species. Collecting eggs and larvae using plankton nets provides a much more economical means of sampling relatively large areas than sampling adults. Ichthyoplankton surveys can thus overcome the problem of limited data on the distribution of a teleost species but requires accurate identification of its early life stages. While H. vittatus larvae 5.3 -29.1 mm SL have been described (Miskiewicz 1987), eggs and larvae <5.3 mm SL are undescribed.

The aim of this paper is to provide a description of the developmental stages of the fertilized eggs and yolk-sac larvae of *H. vittatus* to facilitate the use of ichthyoplankton surveys for determining the spatial distribution of spawning whitebait populations. The embryogeny of *H. vittatus* eggs is only described sufficiently to identify the eggs of this species in plankton collections.

MATERIALS AND METHODS

Commercial catches of whitebait in Western Australia were monitored through 1993. In early August 1993, 12% of a catch of 200–300 kg of whitebait from Warnbro Sound (32°20.8'S, 115°44.5'E) had ripe gonads, suggesting that spawning was probably occurring at this region. On 17 August 1993, plankton tows were completed at several sites within Warnbro Sound, using 60 cm diameter bongo nets with 500 µm mesh, towed just below the surface at 1 m s⁻¹. Fish eggs were particularly abundant at one station close to shore, sampled at 1200 hours. The surface water temperature at this site was 18°C, with a salinity of 35.1. The plankton sample from this tow was taken back to the laboratory, so that fish eggs could be removed and reared.

Culturing of the eggs past hatching was necessary since their identification required identification of the resultant larvae. Although *H. vittatus* larvae <5.3 mm SL have not been described, individuals smaller than this have previously been identified (e.g., Gaughan *et al.* 1990) using Miskiewicz (1987) as a baseline from which to examine a series of sequentially smaller whitebait larvae, which were identified by their elongate shape, the relative length of the gut and the pattern of pigmentation.

At the laboratory, fish eggs were siphoned off from the plankton sample and sorted using a stereomicroscope. Two types of eggs were abundant in the sample. Approximately 100 eggs of one of these types, which exhibited the characteristics of clupeid eggs, were selected for culturing. These characteristics were the spherical shape, the presence of a segmented yolk and a single oil globule (Ahlstrom and Moser 1980; McGowan and Berry 1984). The selected eggs were placed in four glass containers of seawater, two lightly aerated and two non-aerated, and maintained at ambient temperature ($15.2 - 24.0^{\circ}$ C, mean = 17° C). The eggs survived well under these conditions and nearly all them hatched. Forty larvae obtained from these hatched eggs were transferred to a constant temperature tank (18° C). Algae and enriched rotifers were added to the water as a potential food source for the developing larvae.

Samples of eggs and larvae were taken daily and preserved in 5% formalin. The first few reared larvae were identified as being those of *H. vittatus*, thereby confirming that the eggs chosen for culturing were of this species. *Hyperlophus vittatus* eggs were thus also able to be identified. Whilst the sample of eggs collected for rearing did not contain any early embryonic stages, these were described using material collected from previous plankton tows in Warnbro Sound. These earlier stages of whitebait eggs were identified by their size, the presence of a segmented yolk and the relative size of the yolk, as determined from the reared eggs of known identity.

Measurements of eggs and larvae were made using an eyepiece micrometer fitted in a compound microscope. The measured lengths of the larvae refers to body length (BL, tip of snout to tip of the notochord; Leis and Trnski 1989). Descriptions of pigment refers to black pigment (melanin) unless stated otherwise. Because future identification of eggs and early larvae will most likely involve formalin-preserved samples, the illustrations and measurements were done with formalin-preserved material. The illustrations were made with the aid of camera lucida.

RESULTS

Development time of eggs

Most (> 95%) of the reared *H. vittatus* eggs hatched within 48 hours of capture. The seven, least developed eggs had embryonic keels (see Figure 1d) at the time the live sample was sorted (1400 hours, 2 hours after capture). Five of these eggs were allowed to develop through to hatching, which occurred 50 - 51 hours post-capture.

General description of eggs

Hyperlophus vittatus eggs are planktonic and spherical. The eggs have a thin chorion that appears smooth under low magnification. Under high magnification, however, evenly spaced corrugations can be seen. The diameter of formalin-preserved eggs ranges from 0.83 to 0.95 mm, with a mode of 0.93 mm.

Each egg contains one oil globule ranging in diameter from 0.025 to 0.075 mm, with a mode of

0.048 mm. The oil globule is not pigmented.

The yolk is spherical to ovoid, depending on the angle of view (Figure 1a,aa), with a diameter ranging from 0.50 to 0.87 mm (mode = 0.83 mm). In live eggs, the yolk appeared nearly transparent, but became opaque when preserved in formalin and is semi-translucent both under bright transmitted and reflected light. The yolk is coarsely and completely segmented with a 'frothy' or 'bubbly' appearance which is readily apparent during all stages of development.

The perivitelline space occupies 8.6 - 30.9% of the eggs diameter (mode = 14.5%).

Description of various stages during development of the eggs

Initially, the segmented yolk is the most prominent feature of the egg (Figure 1a,aa). Cell growth becomes obvious at the pole opposite to that containing the oil globule, i.e. the vegetative pole (Figure 1 b). This cap of cells appears to bulge slightly over the yolk. The cell-cap flattens out as cells multiply and spread in a thinner layer over the yolk towards the opposite pole (Figure 1c). In contrast to the opacity of the relatively thick cellcap, the yolk is visible beneath this thin layer of cells.

The anterior end of the embryo becomes visible at the vegetative pole as a slightly raised strip (i.e., embryonic keel), with a flatter layer of cells lying at either side (Figure 1d). The embryo is less distinct towards the posterior end and the thin layer of cells, although difficult to observe, is much wider than at the anterior end.

The embryo then becomes more distinct, with the eye and some somites visible (Figure 1e). The tip of the tail lies beyond the oil globule. Divisions of the brain are visible from dorsal view of the head, which lies flat against the yolk. In the majority of the eggs, at this and later stages, the yolk-sac lying beneath the head is concave, but with a central bulge (Figure 1f). However, the yolk remained rounded in a few of the eggs examined (cf. Figures 1f,ff). The concavity may be due to absorption of the yolk by the developing embryo.

With further development, the tail lifts away from the yolk, develops finfolds at the tip and begins to curve to the right. The tail also becomes more pointed and increases in length, while the finfold extends anteriorly almost to the head (Figure 1f). The anterior end of the embryo begins to lift away from the yolk. The gut which is present along the ventral surface of the embryo is difficult to distinguish and has therefore not been shown in Figure 1f.

As development continues, the tail lengthens, the finfold widens and the hindgut becomes more obvious (Figure 1g). At the time of hatching, the dorsoventrally flattened head of the embryo still

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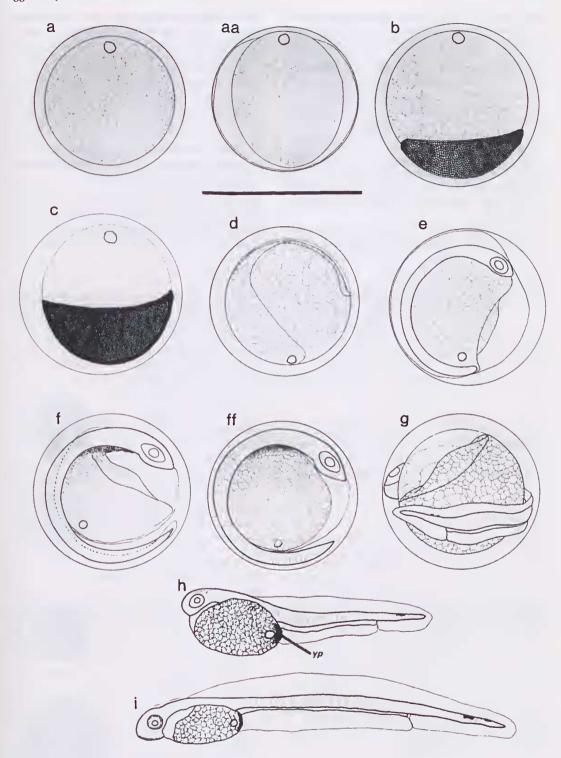


Figure 1 Early life history stages of *Hyperlophus vittatus*. a–g, eggs. h, newly hatched yolk-sac larva, 2.7 mm BL, yp – patch of yellow pigment; i, yolk-sac larvae, 4.2 mm BL. Scale bar equals 1.0 mm.

lies close to the yolk (or yolk-sac membrane), the tail has grown completely around the yolk and overlaps the head, but the mouth has not developed and the eyes are not yet pigmented. The 2-3 small melanophores on the dorsal surface of the tail near the tip of the notochord are characteristic of *H. vittatus* larvae, and whilst illustrated here (Figure 1g), they were often not visible in preserved eggs.

Description of yolk-sac larvae

Newly-hatched larvae were 2.6 mm BL and had typical clupeid characteristics, including a long slender body, a long straight gut and light pigmentation (Figure 1h). The pre-anal length is 70 - 79% BL.

The oil globule is located near the posterior end of the yolk-sac. A patch of yellow pigment, which is often only obvious in fresh or recently preserved specimens, was observed immediately posterior to the oil globule (Figure 1h,i). Newly hatched $H_{...}$ *vittatus* larvae have very fine melanophores scattered over the body, particularly over the head. However, these melanophores are difficult to observe after preservation, so only the denser concentrations on the head have been illustrated. Moreover, they have 2 - 3 distinct melanophores on the dorsal side of the trunk near the notochord tip which are homologous to those observed in well developed embryos within late-stage eggs (cf. Figure 1g).

At the time of hatching, larvae had neither pigmented eyes nor a mouth (Figure 1h). Larvae placed in the constant temperature tank (18°C) had utilised their yolk, developed pigmented eyes and appeared to have a functional mouth after 5 – 7

Table 1The major distinguishing features of the pelagic eggs of clupeids which are common in marine waters of
southern Western Australia. The eggs of each species have segmented yolks. The measures of egg and yolk
diameter for S. sagax from New Zealand (Baker 1972), and egg diameter for E. teres from South Africa
(O'Toole and King 1974) are presented below those recorded in the present study.

Species	Egg diameter (mm) range (mode)	Oil globule	Yolk diameter (mm) range (mode)	Perivitelline space (% of diameter) range (mode)	Diagrammatic representations
Hyperlophus vittatus n = 73	0.83–0.95 (0.93)	yes	0.50–0.87 (0.83)	8.6 - 30.9 (14.5)	
Sardinella lemuru n = 40	1.15–1.32 (1.30)	yes	0.69–0.82 (0.75)	34.2 - 44.8 (39.7)	
Sardinops sagax n = 30	1.34–1.58 (1.44) 1.32–1.70 (1.53) (Baker 1972)	yes	0.62–0.85 (0.69) 0.71–0.83	42.2 - 57.9 (49.4)	
Etrumeus teres n = 52	1.24–1.48 (1.35) 1.32–1.47 (1.37) (O'Toole and King 1974)	no	0.99–1.28 (1.12)	7.1 – 22.6 (18.7)	

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days. They ranged in size from 4.2 to 4.7 mm BL. Rearing of H. vittatus larvae beyond the yolk-sac stage was unsuccessful with the larvae failing to feed on the supplied rotifers and algae. Consequently no larvae survived past 8 days.

DISCUSSION

Eggs

Hyperlophus vittatus eggs which were collected at 1200 hours and already possessed an embryonic keel, required a further 50 – 51 hours to hatch. Since many clupeoid species spawn at night (e.g., Blaxter and Hunter 1982; Sommerton *et al.* 1993), we assumed that the least developed *H. vittatus* eggs collected for culturing had been fertilized between 2000 and 0400 hours the night before capture. Thus, the development time to hatching for *H. vittatus* eggs, at a mean temperature of 17°C, was 58 – 67 hours (2.5 – 3 d).

Hyperlophus vittatus have eggs which are typical of many marine teleosts (Ahlstrom and Moser 1980; Matarese and Sandknop 1984), being spherical with a diameter of about 0.9 mm and possessing a single oil globule. Nonetheless, H. vittatus eggs can be distinguished from those of other species found in plankton collections in southwestern Australia by their size, the segmented yolk, the relative sizes of the yolk and the perivitelline space, and the small oil globule. However, in other regions, the initial identification of H. vittatus eggs should ideally be carried out on the late-stage eggs in which the embryo is well developed. In particular, the 2 - 3 melanophores dorsal of the notochord tip provide a means of identifying late-stage H. vittatus eggs.

The other clupeid species common in southern Western Australian waters are Spratelloides robustus, Sardinella lemuru, Sardinops sagax and Etrumeus teres (Hutchins and Swainston 1986). The eggs of S. robustus have a gelatinous covering and are layed demersally (McGowan and Berry 1984) so would not be expected to occur in plankton samples. The major features which distinguish H. vittatus eggs from those of these other clupeids are the size of the egg and the relative width of the perivitelline space (Table 1). As is the case with all clupeids (McGowan and Berry 1984), the eggs of each of these species have segmented yolks. The dimensions of the eggs of these other species were based on specimens found in plankton samples taken in marine waters off southwestern Australia and preserved in the same way as the H. vittatus eggs used in this study. Baker (1972) provides a full description of S. sagax eggs, while figures of the eggs of several Sardinella species can be found in Bensam (1990). Development of E. teres eggs has been described by O'Toole and King (1974).

Hyperlophus vittatus eggs (diameter of 0.93 mm) are smaller than those of *S. lemuru*¹ (1.30 mm), *S. sagax* (1.44 mm) and *E. teres* (1.35 mm) (Table 1). The perivitelline space occupies 14.5% of the egg diameter in *H. vittatus*, but accounts for 39.7 and 49.4% of the diameter in *S. lemuru* and *S. sagax* respectively. Although the relative size of the perivitelline space in the eggs of *E. teres* (18.7%) is more similar than these other species to that for *H. vittatus*, the eggs of this former species lack an oil globule (Table 1).

In addition to these features, late-stage *H. vittatus* eggs can be identified by the dorsal melanophores on the tail of the embryo and, in fresh specimens, the yellow pigment posterior to the oil globule.

Larvae

The most easily recognisable feature of the yolksac larvae of *H. vittatus*, which can be used to distinguish this species from the other clupeid larvae which occur in southern Western Australia, is the presence of dorsal melanophores near the notochord tip. Yolk-sac larvae of *S. robustus*, *S. lemuru*, *S. sagax* and *E. teres* lack these melanophores. Likewise, the yolk-sac larvae of *Engraulis australis*, which are similar in body form to those of *H. vittatus*, do not possess melanophores dorsal to the notochord tip.

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¹ The eggs of *Sardinella lemuru* have been identified but not formally described.

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